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(54) Title: PROCESS FOR PRODUCING BIOCOMPATIBLE SURFACES

(57) Abstract

The present invention provides an improved process for treating a surface in order to inhibit or prevent the surface from causing blood to clot or coagulate on or at the surface as well as novel compositions used in the practice of the improved process. The present process is particularly adapted for coating polymeric surfaces which directly contact blood and blood products. The process includes sequentially contacting the surface with i) a water soluble polyalkylene amine and a water soluble diepoxide, ii) an anionic compound and iii) a water soluble polyalkylene amine to give a primed surface. The primed surface is then contacted with an antithrombotic agent and a reducing agent to covalently bind the antithrombotic agent to the primed surface.

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PROCESS FOR PRODUCING BIOCOMPATIBLE SURFACES

The present invention generally relates to an improved process for treating a surface in order to inhibit or prevent the surface from causing blood to clot or coagulate on or at the surface as well as novel compositions used in the practice of the improved process. The present process is particularly adapted for coating polymeric or metallic surfaces which directly contact blood and blood products with heparin.

BACKGROUND

Surfaces of medical devices that are in direct contact with blood and blood products have been treated with surface modifying agents, such as heparin, in order to make such surfaces nonthrombogenic. For example, the blood contacting surfaces of devices such as blood oxygenators, blood pumps, catheters, and tubing may be treated with heparin or heparin derivatives to prevent clotting or clot formation related to surface contact with blood or blood products.

Several methods for preparing heparin treated surfaces have been reported. Specifically, U.S. Patent Nos. 4,613,665 and 4,810,784 report a process to attach heparin to substrates. The reported process degrades a polysaccharide antithrombogenic agent such as heparin by diazotation to give fragments which react with primary amino groups of the substrate to form intermediate Schiffs' base conjugates. The intermediate conjugates are then reduced to covalently bind the fragments to the support. This process is limited, in part, because the diazotation step degrades the polysaccharide in order to form the reactive fragments that bond to the substrate.

In addition, U.S. Patent No. 4,565,740 reports a surface modified to include heparin where the substrate is first treated with a polymeric cationic surfactant and a dialdehyde crosslinker, then treated with an anionic compound and finally treated with diazotized heparin and sodium cyanoborohydride. In the first step of the reported process, the cross linking agent is a dialdehyde having from 3-6 carbon

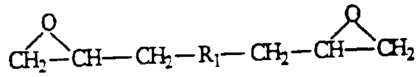
atoms. From this group, glutaraldehyde is expressly identified. This process may be limited because of the use of reagents which may be toxic, hazardous or difficult to handle. For instance, glutaraldehyde is an irritant with a threshold limit value ceiling of 0.2 ppm in air. Similarly, U.S. Patent No. 5,049,403 reports a surface modified to include heparin where the surface is treated with a polyamine and then crosslinked with crotonaldehyde. Crotonaldehyde is also difficult to work with because it is a lacrimator which is highly irritating to eyes, skin, and mucous membranes. Thus, all of the methods referred to above employ agents that require special handling and care which may limit their general applicability.

Further, U.S. Patent No. 4,326,532 reports a layered substrate coated with chitosan which is reacted with an antithrombotic agent such as heparin. The heparin is added to a layer of chitosan which is applied to an acid oxidized or plasma etched hydrophobic surface and then, if needed, the heparin is bonded to the chitosan using sodium cyanoborohydride. The need to etch or acid-oxidize the surface of the substrate also serves to inhibit the general applicability of this process.

A need exits for a process to prepare heparinized surfaces which uses reagents which are easy to handle and are not toxic or hazardous to use. A desirable process would allow the use of biologically active materials without degrading or otherwise altering the biological activity of the active material when it is used to modify the surface of a support. In addition, a need exists for a process which is adapted for use with many different types of surfaces which does not require degradation or alteration of the surface.

SUMMARY OF THE INVENTION

The present invention provides a process to prepare a biocompatible surface which is treated by adding a biologically active or antithrombotic agent to the surface in order to inhibit or prevent blood coagulation. This process includes a priming sequence of contacting a surface with a solution of a water soluble polyalkylene amine having an average molecular weight of between about 1,000-100,000 and a water soluble diepoxide of the general formula



where R_1 is $-O-(CH_2)_a-O-$ and a is 2-3 or $-(O-CH_2-CH_2)_b-O-$ and b is 2-100 in a suitable buffer to give a first primed surface, and contacting the first primed surface with a buffered solution of an anionic compound such as dextran sulfate to give a second primed surface.

After the surface has been primed, a biologically active or antithrombotic agent, preferably heparin, is added to the surface by contacting the second primed surface with a polyalkylene amine having an average molecular weight of between about 1,000-100,000 in a suitable buffer to give a third primed surface and then contacting the third primed surface with a buffered solution of the biologically active or antithrombotic agent and a reducing agent to covalently bind the antithrombotic agent to the primed surface.

One embodiment of this invention includes:

i) contacting a surface with a basic aqueous solution of about 0.001-0.5 wt.%, preferably 0.2 wt.%, polyethylene imine having an average molecular weight of about 50,000-60,000 and about 0.01-1.0 wt.%, preferably 0.1 wt.%, ethylene glycol diglycidyl ether at a temperature of between 20-50°C, preferably room temperature, for about fifteen minutes to give a first primed surface,

ii) contacting the first primed surface with an acidic aqueous solution of about 0.001-0.1 wt.%, preferably 0.03 wt.%, dextran sulfate at a temperature of between 20-50°C, preferably room temperature, for about five minutes to give a second primed surface,

iii) contacting the second primed surface with a basic aqueous solution of about 0.001-0.5 wt.%, preferably 0.2 wt.%, polyethylene imine having an average molecular weight of about 50,000-60,000 at a temperature of about 20-50°C for about five minutes to give a third primed surface, and

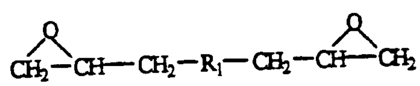
iv) contacting the third primed surface with an acidic aqueous solution of about 0.01-1.0 wt.%, preferably 0.04 wt.%, periodate oxidized heparin and

0.001-1.0 wt.%, preferably 0.004 wt.%, sodium cyanoborohydride at a temperature of about 50°C for about 0.5-24 hours, preferably 2 hours to give a biocompatible surface.

Preferably, the surface is thoroughly rinsed with deionized water between each of the above listed steps i-iv. Steps i and ii may be repeated as needed. The basic aqueous solutions in steps i and iii may be a basic buffer solution such as carbonate or borate which provides a pH of about 9. A suitable basic buffer solution is 0.3 wt.% borate buffer at pH 9. The acidic aqueous solutions in steps ii and iv may be an acidic buffer such as a phosphate or citrate buffer which provides a pH of about 3-4.2. A suitable acidic buffer solution is 1 wt.% citrate buffer and 0.9 wt.% suitable sodium chloride at pH 3-4, preferably pH 3.9.

Surfaces which are particularly well suited for use in the present process include polymeric surfaces such as cellulose, poly(vinyl alcohol), poly(vinyl chloride), poly(methylmethacrylate), polycarbonate, polyurethane, polypropylene, polysulfone, poly(tetrafluoroethylene), silicone, and poly(ethylene terephthalate) as well as other surfaces typically used in medical devices such as glass and stainless steel.

The above described process is particularly suited to add a novel four part biocompatible coating composition to surfaces that will contact blood or blood products. The coating composition includes a water soluble polyalkylene amine crosslinked with a water soluble diepoxide, an anionic compound such as dextran sulfate, a polyalkylene amine, and a biologically active or antithrombotic agent. In this coating composition, preferred polyalkylene amines have an average molecular weight of between about 1,000-100,000 and, more preferably, between about 20,000-60,000. A particularly preferred polyalkylene amine is polyethylene imine having an average molecular weight of about 50,000-60,000. Preferred diepoxides are selected from compounds of the general formula



where R_1 is $-O-(CH_2)_a-O-$ and a is 2-3 or $-(O-CH_2-CH_2)_b-O-$and b is 2-100. A particularly preferred diepoxide is ethylene glycol diglycidyl ether. Preferred biologically active or antithrombotic agents include biologically active oligo or polysaccharides containing glucosamine or galactosamine subgroups such as heparin, heparan sulfate, hyaluronic acid, dermatan sulfate, chitosan and active derivatives of these polysaccharides. A particularly preferred antithrombotic agent is heparin or a derivative thereof.

When the present coating composition is compared with other coating compositions that use aldehyde-containing crosslinking agents, it was unexpectedly found that the present composition provides a surface which is smoother and more uniform than the aldehyde-cross linked compositions. A smoother, more uniform surface may be desirable because smooth, uniform surfaces are less likely to promote blood clot or coagulation as compared to a rough, irregular surface of the same material.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a scanning electron microscope photomicrograph of a polycarbonate surface treated with a coating composition of the present invention.

Figure 2 is a scanning electron microscope photomicrograph of a poly(methylmethacrylate) surface treated with a coating composition of the present invention.

Figure 3 is a scanning electron microscope photomicrograph of a polycarbonate surface treated with a coating composition containing a glutaraldehyde cross linking agent.

Figure 4 is a scanning electron microscope photomicrograph of a poly(methylmethacrylate) surface treated with a coating composition containing a glutaraldehyde cross linking agent.

Figure 5 is a scanning electron microscope photomicrograph of a polycarbonate surface treated with a coating composition containing a crotonaldehyde cross linking agent.

Figure 6 is a scanning electron microscope photomicrograph of a poly(methylmethacrylate) surface treated with a coating composition containing a crotonaldehyde cross linking agent.

DETAILED DESCRIPTION

The present invention provides a process to produce biocompatible or blood compatible surfaces. As used in this specification, a "biocompatible or blood compatible surface" is a treated surface which, when in contact with a patient's blood, plasma, or other body fluids, does not cause an adverse physiological reaction. Treated surfaces preferably include a "biologically active or nonthrombotic agent" is a material which, when in contact with a patient's blood, plasma, or other body fluids under physiological conditions, exhibits biological activity. For example, heparin is biologically active or nonthrombogenic in the sense that it acts as an anti-coagulant in the presence of blood.

According to this invention, both hydrophobic and hydrophilic surfaces are first contacted with a mixture of a polyalkylene amine such as polyethylene imine and a diepoxide crosslinking agent such as ethylene glycol diglycidyl ether to give a surface having an outer layer which is both wettable and positively charged. Next, an anionic compound or negatively charged agent such as dextran sulfate is added to the positively charged surface to give an outer layer which further increases the wettability of the surface and also allows the addition of another layer of polyamine to the surface. Thus, the surface is treated or primed by the sequential treatment with three agents: i) polyalkylene amine and crosslinker, ii) dextran sulfate, and iii) polyalkylene amine.

To complete the process, the treated surface is then contacted with an antithrombotic agent such as heparin in the presence of sodium cyanoborohydride which covalently binds the agent to the polyalkylene amine. The covalent binding of the biologically active or antithrombotic agent to the polyalkylene amine most likely occurs when a reactive terminal aldehyde group of the antithrombotic agent reacts with a primary amino group of the polyamine. The initial Schiff's base

intermediate is readily reduced to a secondary amine in the presence of a reducing agent such as sodium cyanoborohydride.

Alternatively, the treated surface may be contacted a biologically active or nonthrombotic agent having free aldehyde groups (generated, e.g., by periodate oxidation) in the presence of a reducing agent such as sodium cyanoborohydride which also covalently binds the agent to the polyalkylene amine. The covalent binding of the biologically active agent to the polyalkylene amine most likely occurs when a reactive aldehyde group of the biologically active agent reacts with a primary amino group of the polyalkylene amine. The Schiffs' base initially formed as a result is readily reduced to a secondary amine in the presence of the sodium cyanoborohydride.

In yet another embodiment, covalent binding of the biologically active or nonthrombotic agent may also be accomplished using a carbodiimide coupling agent, rather than sodium cyanoborohydride, in which case it is not necessary to use periodate oxidation to generate free aldehyde groups.

EXAMPLES

The following examples are provided to further illustrate the practice of the present invention. The examples are not intended to limit the invention which is defined in the appended claims.

In the examples, the surface-bound concentration of heparin may be measured by a thrombin inhibition assay. The inhibition assay exploits the observation that thrombin enzymatically cleaves a synthetic substrate (S-2238) to yield a product whose concentration is proportional to its absorbance at 405 nm, and the concentration of product is therefore proportional to the thrombin concentration. Decreased amounts of product reflect inhibition of thrombin by heparin in the presence of excess amounts of antithrombin-III. Briefly, the assay is performed by adding, in the following sequence, the listed materials to test tubes: an unknown sample and 0.05 ml of buffer (where the sample has unknown concentration of heparin on the surface), or 0.05 ml of a standard heparin solution, 1.0 ml of 0.3 mM S-2238, 0.1 ml of antithrombin-III (5 units/ml), and 0.1 ml of

thrombin (0.1 units/ml). The standard heparin solutions (50 microliters) contain, for example, 0.08, 0.04, 0.02, 0.01 and 0.0 micrograms of heparin. The assay is carried out at 37°C with overnight incubation in a water bath, with continuous mixing. Measurements are made on 0.20 ml aliquots taken from the unknown and standard solutions using microtiter plates and optical density at 405 nm is recorded. The optical density values are related to heparin concentration using the heparin standard solutions.

More specifically, the assay procedure is a modification of Chandler et al., *J. Biomed. Mater. Res.*, 22:497-508 (1988) which uses the following reagents.

Reagent	Manufacturer	Concentration
Antithrombin-III	Sigma	5 units/ml
S-2238	Kabi	0.3 mM
Thrombin	Sigma	5 units/ml
Hanks' Buffer	Sigma	0.1 units/ml
Heparin	Sigma	10 units/ml

Antithrombin-III is reconstituted to 5 units/ml with 10 ml deionized distilled water and refrigerated at 4°C. S-2238 is reconstituted to 0.3 mM using 133 ml of buffer stock solution of PBS (phosphate buffered saline) with 1 mg/ml BSA (bovine serum albumin, Cat. No. A7838, Sigma Chemical Company, St. Louis, MO) and 1 mg/ml polyethylene glycol (8000 MW, Cat. No. P2139, Sigma Chemical Company, St. Louis, MO) and stored at 4°C. Thrombin is reconstituted to 10 units/ml with 10 ml Hanks' phosphate buffered saline and stored at -20°C in 1 ml aliquots. A 1:100 dilution of thrombin is used in the assay.

Standard heparin solutions are prepared from the 10 units/ml stock solution by serial dilution. Each new batch of thrombin and/or heparin must be tested to insure maximum sensitivity. Representative values of standard heparin solutions are listed in the following table.

Standard	Concentration
1	.08 mg/50 ml
2	.04 mg/50 ml
3	.02 mg/50 ml
4	.01 mg/50 ml
5	0 mg

To measure absorbence, 0.05 ml of appropriate standards as well as an unknown sample having a measured surface area together with PBS/BSA buffer (0.05 ml) are dispensed into tubes. The following reagents are added to each of the tubes, 0.1 ml antithrombin-III, 1.0 ml S-2238 and 0.1 ml thrombin, all tubes are vortexed and then incubated overnight at 37°C. After incubation, 0.2 ml from each tube is added to a well of a microtiter plate in duplicate for each tube and optical density readings are taken at 405 nM. (All standards and samples are run in duplicate, with duplicate optical density readings at 405 nM.)

Example 1 - Surface Priming

Polycarbonate (PC, HYZOD, Sheffield Plastics Inc., Sheffield, MA) sheets were treated with polyethylene imine having an average molecular weight of 50,000 (PEI, 500 mg, Aldrich Chemical Company, Milwaukee, WI) and ethylene glycol diglycidyl ether (EGDE, 500 mg, Aldrich Chemical Company, Milwaukee, WI) in 0.3% borate buffer at pH 9 for fifteen minutes at room temperature and were then treated with a solution of dextran sulfate (100 mg, 500,000 MW, Sigma Chemical Company, St. Louis, MO) in 1% citrate buffer and 0.9 wt.% sodium chloride at pH 3 for five minutes at 50°C. The treated sheets stained purple with toluidine blue which indicates the presence of the dextran sulfate.

Contact angle measurements using the sessile drop technique done on the treated PC sheet after the above steps demonstrated the water wetting ability of this process.

TABLE 1

Contact Angle Measurements on Polycarbonate at Various Stages of Priming	
Surface	Contact Angle (sessile drop)
PC untreated	81.0 ± 1.0
PC after PEI step	42.8 ± 5.8
PC after DS step	21.9 ± 12.7

<u>Example 2 - Heparin Attachment to Surfaces</u>

Various substrates listed in Table 2 were primed with a borate buffer solution (4.6 g sodium borate decahydrate in one liter water, adjusted to pH 9 with sodium hydroxide) of polyethylene imine (PEI, average molecular weight 50,000, Aldrich Chemical Company, Milwaukee, WI) and ethylene glycol diglycidyl ether (EGDE, Aldrich Chemical Company, Milwaukee, WI) followed by a citrate buffer solution (11.0 g citric acid monohydrate and 9.0 g sodium chloride in one liter water, adjusted to pH 3.0 with 5N sodium hydroxide) of dextran sulfate (average molecular weight 500,000, Sigma Chemical Company, St. Louis, MO) using the amount of reagents and reaction time and temperatures listed in Table 2 (in the table r.t. means room temperature). After each of the priming steps, the surfaces were thoroughly rinsed with deionized water.

The primed surfaces were than treated with polyethylene imine in a borate buffer solution in the amounts and under the conditions listed in Table 2, rinsed thoroughly with deionized water, and then contacted with sodium heparin (Diosynth Inc., Chicago, IL) and sodium cyanoborohydride (Aldrich Chemical Company, Milwaukee, WI) in additional citrate buffer (the buffer was prepared as described above except that the pH is adjusted to 3.9).

After rinsing with water, the treated surfaces were tested for heparin activity using the thrombin inhibition assay (described above). The thrombin inhibition assay data is also listed in Table 2.

TABLE 2

SUBSTRATE	PEI/EGDE	DEXTRAN SULFATE	PEI	HEPARIN/ CYANOBOROHYDRIDE	HEPARIN ACTIVITY ($\mu g/cm^2$)
Poly(methyl methacrylate) PLEXIGLASS	330 ml 0.3% borate buffer 33 mg PEI 330 mg EGDE 5 min, r.t.	500 ml pH 3.0 citrate buffer 150 mg dextran sulphate 5 min., 50°C	420 ml 0.3% borate buffer 168 mg PEI 5 min., r.t.	1000 ml pH 3.9 citrate buffer 2.5 g sodium heparin 250 mg sodium cyanoborohydride 4 hr., 50°C	0.22
Porous polypropylene CELGARD					0.10
Stainless steel 304 stainless steel	300 ml 0.3% borate buffer 33 mg PEI 500 mg EGDE 30 min., 25°C	500 ml pH 3.0 citrate buffer 150 mg dextran sulphate 10 min., 50°C	500 ml 0.3% borate buffer 168 mg PEI 5 min., r.t.	1000 ml pH 3.8 citrate buffer 5.0 g sodium heparin 1.0 g sodium cyanoborohydride 4 hr., 50°C	0.12
Poly(vinyl chloride)	330 ml 0.3% borate buffer 33 mg PEI 330 mg EGDE 15 min., r.t.	500 ml pH 3.0 citrate buffer 150 mg dextran sulfate 5 min., 50°C	420 ml 0.3% borate buffer 168 mg PEI 5 min., r.t.	1000 ml pH 3.9 citrate buffer 2.5 g sodium heparin 250 mg sodium cycanoborohydride 4 hr., 50°C	•
Polycarbonate HYZOD					0.15
Polycarbonate HYZOD	300 ml 0.2 wt% PEI/0.1 wt% EGDE 15 min., r.t.	500 ml pH 3.9 citrate buffer 150 mg dextran sulfate 5 min., r.t.	300 ml 0.2 wt% PEI 15 min., r.t.	1000 ml pH 3.9 citrate buffer 2.5 g sodium heparin 250 mg sodium cyanoborohydride 4 hr, 50 °C	0.11
Poly(tetrafluoroethylene) TEFLON	500 ml 0.3% borate buffer 33 mg PEI 500 mg EGDE 30 min., r.t.	500 ml pH 3.0 citrate buffer 100 mg dextran sulfate 5 min., 50°C	500 ml 0.3% borate buffer 168 mg PEI 5 min., r.t.	1000 ml pH 3.8 citrate buffer 2.5 g sodium heparin 250 mg sodium cycanoborohydride 4 hr., 50°C	0.26
Poly(ethylene terephthalate) SCOTCHPAR					0.35
Silicone SILASTIC		500 ml pH 3.0 citrate buffer 100 mg dextran sulfate 15 min., 50°C		1000 ml pH 3.8 citrate buffer 5.0 g sodium heparin 1.0 g sodium cyanoborohydride 120 hr., 50°C	0.12
Polyurethane HEMOTHANE					•
Polysulfone THERMALUX					0.26

TABLE 2 (cont.)

SUBSTRATE	PEI/EDGE	DEXTRAN SULPHATE	PEI	HEPARIN/ CYANOBOROHYDRIDE	HEPARIN ACTIVITY ($\mu g/cm^2$)
Poly(vinyl alcohol) prepared according to procedures reported in U.S. 4,428,325	330 ml 0.3% borate buffer 33 mg PEI 165 mg EGDE 30 min., 50°C	500 ml pH 3.0 citrate buffer 100 mg dextran sulfate 10 min., 50°C	600 ml 0.3% borate buffer 168 mg PEI 10 min., r.t.	1000 ml pH 3.8 citrate buffer 5.0 g sodium heparin 1.0 g sodium cyanoborohydride 3 hr., 50°C	0.14

*presence of heparin determined only by staining with toluidine blue

<u>Material Sources for Substrates of Table 2</u>

PLEXIGLASS, Rohm and Haas, Philadelphia, PA
CELGARD, Hoechst Celanese, Charlotte, NC
HYZOD, Sheffield Plastics Inc., Sheffield, MA
SCOTCHPAR, 3M Company, St. Paul, MN
SILASTIC, Dow Corning, Midland, MI
TEFLON, Zeus Industrial Products Inc., Raritan, NJ
HEMOTHANE, 3M, St. Paul, MN
THERMALUX, The Westlake Companies, Redding, PA
Poly(vinyl chloride), Nalgene Company, Rochester, NY

Example 3 - Surface Treated Oxygenator

A Sarns/3M adult membrane oxygenator (made of polypropylene, polycarbonate, and stainless steel, Model 16310, Sarns/3M, Ann Arbor, MI) as well as the related centrifugal pump (made of polycarbonate and poly(methyl methacrylate), Model 7850, Sarns/3M, Ann Arbor, MI), reservoir bag and tubing (both made of poly(vinyl chloride), Sarns/3M, Ann Arbor, MI) were treated according to the procedure of Example 2, above, to modify the blood contacting surfaces of each part of the system using conditions reported in Table 2 for the treatment of poly(vinyl chloride) and polycarbonate.

After treating each of the blood-contacting surfaces of the system, the complete system was rinsed with phosphate buffered saline (pH 7) for two hours at 37° to remove ionically bound heparin. Without this step, it was found that ionically bound heparin was eluted into the circulating blood during testing.

Qualitative blood compatibility was tested using a porcine animal model by maintaining blood flow through the treated system for a period of three hours. During this time, no heparin was administered to the animal and no heparin was eluted into the circulation as determined by monitoring coagulation times of the animal's blood.

At the end of the initial three hour period, the surface treated oxygenator was replaced with an oxygenator that had no surface treatment. After an additional three hour period, the entire system was disassembled and visually examined for the presence of thrombi. The surface treated oxygenator showed no signs of thrombi except for a few small thrombi on the stainless steel heat exchanger in the oxygenator. In comparison, the substantially identical oxygenator that had not been treated as described above exhibited massive thrombi formation on all parts of the untreated oxygenator.

Example 4 - Surface Treated Catheter

An INSYTE twenty gauge polyurethane catheter (Deseret Medical Inc./Becton Dickenson and Company, Sandy, UT) was treated with the reagents listed in Table 2 for a polyurethane substrate using the listed times and temperatures

with the exception that the catheter was treated with the buffered solution of sodium heparin and sodium cyanoborohydride for four hours. Upon completion of the surface treatment process, the catheter was thoroughly rinsed with water. The presence of heparin on the catheter surface was determined by staining with toluidine blue.

Example 5 - Comparative Example

In this example, polycarbonate and poly(methylmethacrylate) surfaces were primed with three different coating compositions according to the procedures generally described in Example 2 except that polyethylene imine alone and heparin were not added.

Briefly, a polycarbonate or poly(methyl methacrylate) surface was primed or treated using a three step process. The surface was contacted with a buffered solution of polyethylene imine and a cross linking agent, then with a buffered solution of dextran sulphate and finally with a second buffered solution of polyethylene imine and cross linking agent. Three different cross linking agent were used, ethylene glycol diglycidyl ether (EGDE), glutaraldehyde (GA, see U.S. Patent 4,565,740)), or crotonaldehyde (CTA, see U.S. Patent 5,049,403). Reaction times, buffers and concentrations are listed in Table 3.

TABLE 3

Primer with EGDE cross linking agent	Polycarbonate reaction time/temp.	Poly(methylmethacrylate) reaction time/temp.
0.005 wt% PEI/0.1 wt% EGDE in borate buffer	15 min./r.t.	5 min./r.t
0.03 wt% Dextran Sulfate in citrate buffer	5 min./50°C	5 min./50°C
0.005 wt% PEI/0.1 wt% EGDE in borate buffer	15 min./r.t.	5 min./r.t.
Primer with GA cross linking agent		
0.005 wt% PEI/0.5 wt% GA in borate buffer	15 min./r.t.	5 min./r.t.
0.03 wt% Dextran Sulfate in citrate buffer	5 min./50°C	5 min./50°C
0.005 wt% PEI/0.5 wt% GA in borate buffer	15 min./r.t.	5 min./r.t.
Primer with CTA cross linking agent		
0.005 wt% PEI/0.034 wt% GA in borate buffer	15 min./r.t.	5 min./r.t.
0.03 wt% Dextran Sulfate in citrate buffer	5 min./50°C	5 min./50°C
0.005 wt% PEI/0.034 wt% GA in borate buffer	15 min./r.t.	5 min./r.t.

PEI - polyethylene imine
EGDE - ethylene glycol diglycidyl ether
GA - glutaraldehye
CTA - crotonaldehyde, borate buffer pH 9, citrate buffer pH 3.9
r.t. - room temperature

After priming, the treated surfaces were examined under a scanning electron microscope. Representative views of the various surfaces are illustrated in Figs. 2-6. The photomicrographs indicate that using EGDE as a cross linking agent provided a surface which was different when compared with the surfaces provided when GA or CTA were used as cross linking agents. The EGDE-containing surface was smoother and more uniform compared to the other surfaces.

It is believed that the particulate material observed in the photomicrographs of GA-containing or CTA-containing polycarbonate or poly(methylmethacrylate) surfaces is related to a suspension polymerization phenomena. Both GA and CTA react rapidly with amines and may also self-polymerize which may result in the formation of reactive particulate material in the buffered solutions. The particulate material is then deposited on the surface. In a similar experiment polycarbonate surfaces were contacted with the above solutions for five minutes. When the

contact time was shorter the amount of observed particulate material was less for the GA-containing surface and not seen for the CTA-containing surface. These observations indicate that the particulate formation may also be time as well as surface dependant.

In contrast to GA and CTA, EGDE is not as reactive and this agent does not self-condense or self-polymerize. As a result, EGDE-containing solutions do not form particulate material and the photomicrographs of the surfaces treated with EGDE-containing solutions illustrate that the resulting surface was comparatively smoother and more uniform.

Example 6 - Oxidized Heparin Attachment to Surfaces

Various substrates listed in Table 4 were immersed in an aqueous solution of polyethylene imine (PEI 0.40 g/400 ml distilled water, average molecular weight 60,000, Aldrich Chemical Company, Milwaukee, WI) and ethylene glycol diglycidyl ether (EGDE 0.40 g/400 ml distilled water, Aldrich Chemical Company, Milwaukee, WI) for fifiteen minutes at room temperature and then rinsed thoroughly with water. The substrates were then immersed in a citrate buffer solution (11.0 g citric acid monohydrate and 9.0 g sodium chloride in one liter water, adjusted to pH 3.0 with 5N sodium hydroxide) of dextran sulfate (0.15 g/ 500 ml citrate buffer, molecular weight 500,000, Sigma Chemical Company, St. Louis, MO) for five minutes and then again rinsed thoroughly with water.

Following dextran sulfate treatment, the substrates then immersed in in an aqueous PEI solution (PEI 0.40 g/400 ml distilled water, average molecular weight 60,000, Aldrich Chemical Company, Milwaukee, WI) for fifteen minutes at room temperature. Following a thorough rinsing with water, the substrates were immersed in a solution containing 0.04% by weight periodate oxidized heparin and 0.004% by weight sodium cyanoborohydride (Aldrich Chemical Co., Milwaukee, WI) in the above-described citrate buffer for two hours at 50°C.

Periodate oxidized heparin was prepared by dissolving sodium heparin (15 g, Diosynth Inc., Chicago, IL) and sodium periodate (1.5 g) in phosphate buffered saline (450 ml, pH 7), and then stirring the solution in the dark for one

hour. Glycerin (15 g) was then added to quench the unreacted periodate, after which the mixture was stirred for one hour and then dialyzed against water (4 times, using a total of 4 liters of water) using 1000 MWCO dialysis tubing. The dialyzed solution was then lyophilized to yield about 8 g of periodate oxidized heparin.

Following exposure to the periodate oxidized heparin/sodium cyanoborohydride solution, the sample was rinsed thoroughly with water, with 25% saline solution for ten minutes at room temperature to remove any ionically bound heparin and then finally rinsed thoroughly with water. Each substrate was tested for heparin activity using the thrombin inhibition assay (described above). The heparin activity results (in $\mu g/cm^2$) are shown in Table 4. In addition, the presence of heparin on all surfaces was confirmed by staining with toluidine blue.

TABLE 4

SUBSTRATE	HEPARIN ACTIVITY ($\mu g/cm^2$)
PLEXIGLASS Poly(methyl methacrylate)	0.07
CELGARD Porous polypropylene	0.08
304 stainless steel	0.17
Poly(vinyl chloride)	*
HYZOD Polycarbonate	0.08
TEFLON Poly(tetrafluoroethylene)	0.10
SCOTCHPAR Poly(ethylene terephthalate)	0.06
SILASTIC Silicone	0.19
HEMOTHANE Polyurethane	*
THERMALUX Polysulfone	0.07

*presence of heparin determined only by staining with toluidine blue

<u>Material Sources for Substrates of Table 4</u>

PLEXIGLASS, Rohm and Haas, Philadelphia, PA
CELGARD, Hoechst Celanese, Charlotte, NC
HYZOD, Sheffield Plastics Inc., Sheffield, MA
SCOTCHPAR, 3M Company, St. Paul, MN
SILASTIC, Dow Corning, Midland, MI
TEFLON, Zeus Industrial Products Inc., Raritan, NJ
HEMOTHANE, 3M, St. Paul, MN
THERMALUX, The Westlake Companies, Redding, PA
Poly(vinyl chloride), Nalgene Company, Rochester, NY

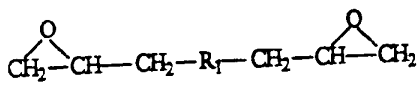
Example 7 - Surface Treated Oxygenator

An oxygenator circuit as described in Example 3 was treated using the procedure described in Example 6 except that the rinse was 25% saline solution was omitted. This oxygenator circuit was tested as in Example 3. During this time no heparin was administered to the animal and no heparin eluted from the oxygenator circuit as determined by monitoring coagulation times of the animal's blood during the experiment. At the end of the three hour period the surface treated oxygenator and heat exchanger were nearly thrombus free. The remaining parts of the circuit were thrombus free.

CLAIMS

1. A process to prepare a biocompatible surface by adding an antithrombotic agent to the surface in order to inhibit or prevent blood coagulation comprising the steps of

i) contacting the surface with a solution of a water soluble polyalkylene amine having an average molecular weight of between about 1,000-100,000 and a water soluble diepoxide of the formula



where R_1 is $-O-(CH_2)_a-O-$ and a is 2-3 or $-(O-CH_2-CH_2)_b-O-$ and b is 2-100 to give a first primed surface,

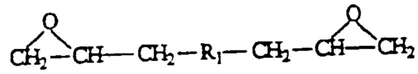
ii) contacting the first primed surface with a solution of an anionic compound to give a second primed surface,

iii) contacting the second primed surface with a solution of a polyalkylene amine having an average molecular weight between about 1,000-100,000 to give a third primed surface, and

iv) contacting the third primed surface with a solution of antithrombotic agent and a reducing agent to covalently bind the antithrombotic agent to the primed surface.

2. A process to prepare a biocompatible surface by adding an antithrombotic agent to the surface in order to inhibit or prevent blood coagulation comprising the steps of:

i) contacting the surface with a basic aqueous solution of about 0.001-0.5 wt.% of a water soluble polyalkylene amine having an average molecular weight of between about 1,000-100,000 and about 0.01-1.0 wt.% of a diepoxide of the formula



where R_1 is $-O-(CH_2)_a-O-$ and a is 2-3 or $-(O-CH_2-CH_2)_b-O-$and b is 2-100 at pH 9 at a temperature of between 20-50°C for about fifteen minutes to give a first primed surface,

ii) contacting the first primed surface with an acidic aqueous solution of 0.001-0.1 wt.% dextran sulfate at pH 3-4 at a temperature of between 20-50°C for about five minutes to give a second primed surface,

iii) contacting the second primed surface with a basic aqueous solution of about 0.001-0.5 wt.% of a water soluble polyalkylene amine having an average molecular weight between about 1,000-100,000 at a temperature of about 20-50°C for about five minutes to give a third primed surface, and

iv) contacting the third primed surface with an acidic aqueous solution of 0.01-1.0 wt. % antithrombotic agent and 0.001-1.0 wt. % sodium cyanoborohydride at a temperature of about 50°C for about 0.5-24 hours to give a biocompatible surface.

3. A biocompatible coating composition comprising

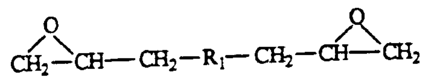
i) a water soluble polyalkylene amine having an average molecular weight of between about 1,000-100,000 crosslinked with a water soluble diepoxide,

ii) dextran sulfate,

iii) polyalkylene amine having an average molecular weight of between about 1,000-100,000, and

iv) an antithrombotic agent.

4. The composition of claim 3 wherein the water soluble diepoxide is selected from the group consisting of compounds of the formula



where R_1 is $-O-(CH_2)_a-O-$ and a is 2-3 or $-(O-CH_2-CH_2)_b-O-$ and b is 2-100.

5. The composition of claim 3 wherein the antithrombotic agent is selected from the group consisting of heparin, heparan sulfate, hyaluronic acid, dermatan sulfate, chitosan and derivatives thereof.

6. A medical article having a nonthrombogenic surface comprising a biocompatible substrate and an antithrombogenic coating comprising

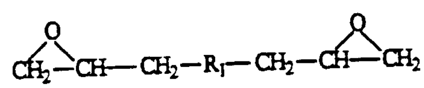
i) a water soluble polyalkylene amine having an average molecular weight of between about 1,000-100,000 crosslinked with a water soluble diepoxide,

ii) dextran sulfate,

iii) polyalkylene amine having an average molecular weight of between about 1,000-100,000, and

iv) an antithrombotic agent.

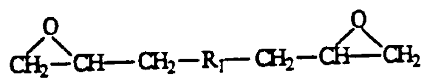
7. The medical article of claim 6 wherein the water soluble diepoxide is selected from the group consisting of compounds of the formula



where R_1 is $-O-(CH_2)_a-O-$ and a is 2-3 or $-(O-CH_2-CH_2)_b-O-$ and b is 2-100.

8. The article of claim 7 wherein the antithrombotic agent is selected from the group consisting of heparin, heparan sulfate, hyaluronic acid, dermatan sulfate, chitosan and derivatives thereof, and wherein the surface is selected from the group consisting of cellulose, poly(vinyl alcohol), poly(vinyl chloride), poly(methylmethacrylate), polycarbonate, polyurethane, polypropylene, polysulfone, poly(tetrafluoroethylene), silicone, poly(ethylene terephthalate), glass and stainless steel.

9. In a process for preparing a biocompatible surface by covalently binding an antithrobmotic agent to a polyalkylene amine on the surface wherein the improvement comprises priming the surface with a solution of a water soluble polyalkylene amine and a water soluble diepoxide of the formula



where R_1 is $-O-(CH_2)_a-O-$ and a is 2-3 or $-(O-CH_2-CH_2)_b-O-$and b is 2-100.

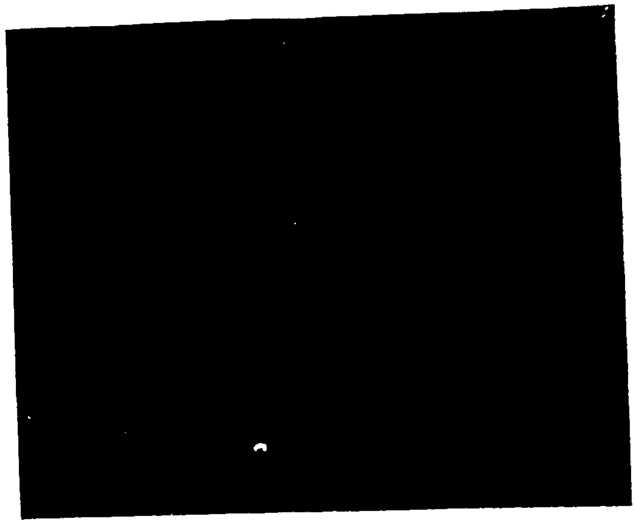


Fig. 1

Fig. 2

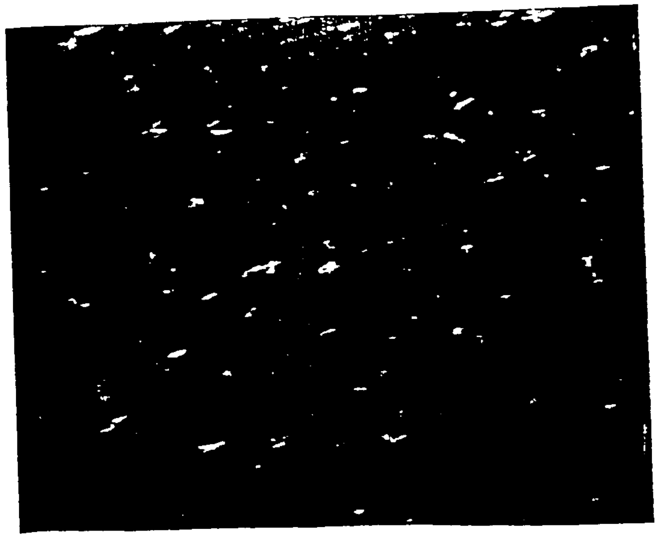


Fig. 3

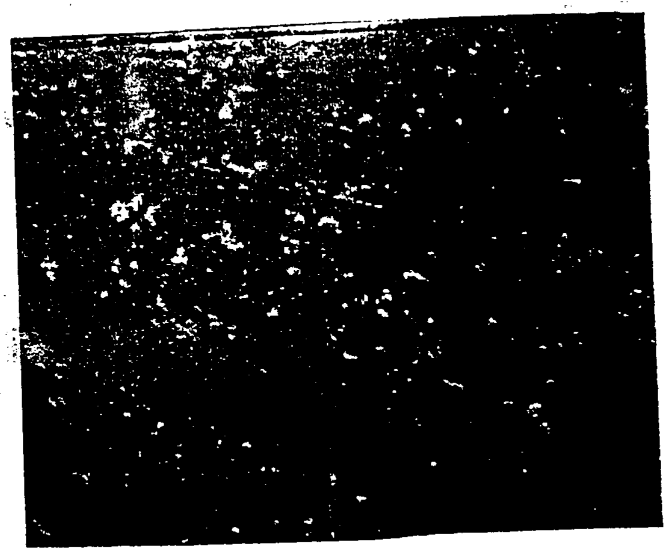


Fig. 4

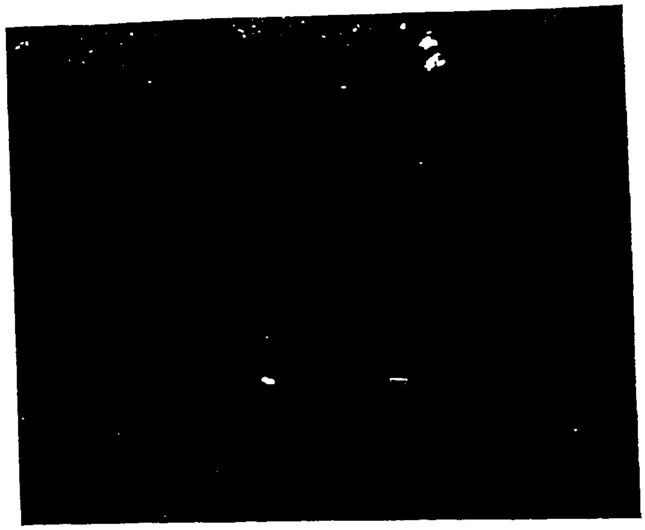


Fig. 5

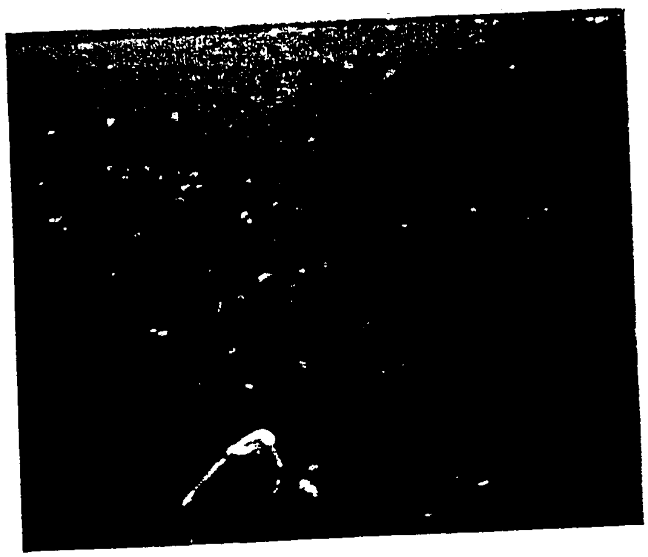


Fig. 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/05893

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L33/00 C08J7/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO,A,91 05817 (NORSK HYDRO AS) 2 May 1991 see claims & US,A,5 049 403 cited in the application ---	1-9
A	EP,A,0 086 187 (IRD BIOMATERIAL AB) 17 August 1983 see claims; examples & US,A,4 565 740 cited in the application ---	1-9
A	EP,A,0 124 676 (IRD BIOMATERIAL AB) 14 November 1984 see claims --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
30 September 1996	09. 10. 96

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/05893

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members

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